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THE DIFFERENCES IN THE EFFECTS OF PROPOLIS, SODIUM HYPOCHLORITE, AND EDTA AS IRRIGANT SOLUTIONS ON THE MICROHARDNESS OF ROOT CANAL DENTIN: *IN-VITRO* STUDY

(PERBEDAAN EFEK PROPOLIS, SODIUM HIPOKLORIT, DAN EDTA SEBAGAI BAHAN IRIGASI TERHADAP KEKERASAN MIKRO DENTIN SALURAN AKAR SECARA IN-VITRO)

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ABSTRACT

Sodium hypochlorite (NaOCl) and EDTA are commonly used irrigants due to their antimicrobial properties and ability to dissolve organic and inorganic components. However, using these chemicals at varying concentrations and durations can affect root canal dentin's physical and chemical properties. Recently, propolis has gained attention as an alternative irrigant because it shows similar potential to conventional irrigants. This study aims to examine whether there are differences in the effects of propolis extract, NaOCl, and EDTA on the microhardness of root canal dentin. This study utilized 24 single-rooted premolar teeth. The crowns were removed, and the roots were longitudinally split into two halves. Samples were randomly divided into six

groups (n=8), each immersed in 8% propolis, 20% propolis, 30% propolis, 2.5% NaOCl, 5% NaOCl, and 17% EDTA. The microhardness of root canal dentin was measured using a Vickers Hardness Tester before and after immersion. Data were analyzed using One-way ANOVA. The results showed that 8% propolis had the most minor decrease in microhardness among all treatment groups, with an average difference in initial and final microhardness of 3.68, followed by 20% propolis, 2.5% NaOCl, 5% NaOCl, 30% propolis, and 17% EDTA. This study concludes that there are significant differences ($p<0.05$) among the treatment groups regarding the microhardness of root canal dentin. Extracts of 8% and 20% propolis can be used as alternative irrigants.

Keywords: irrigants; EDTA; microhardness; NaOCl; propolis

ABSTRAK

Sodium hipoklorit (NaOCl) dan EDTA adalah bahan irigasi yang umum digunakan karena memiliki sifat antimikrobial serta kemampuan keduanya dalam melarutkan komponen organik dan anorganik. Namun, penggunaan bahan kimia ini dengan konsentrasi dan durasi yang bervariasi dapat mempengaruhi sifat fisik maupun kimia yang dimiliki oleh dentin saluran akar. Belakangan ini, propolis telah banyak digunakan sebagai bahan irigasi alternatif karena memiliki potensi yang hampir sama dengan bahan irigasi yang umum digunakan. Tujuan dari penelitian ini adalah untuk melihat apakah terdapat perbedaan efek antara ekstrak propolis, NaOCL dan EDTA terhadap kekerasan mikro dentin saluran akar. Penelitian ini menggunakan 24 gigi premolar berakar tunggal. Mahkotanya dipotong dan akarnya dibelah secara longitudinal menjadi dua. Sampel dibagi secara acak menjadi 6 kelompok (n=8) masing-masing direndam dengan propolis 8%, propolis 20%, propolis 30%, NaOCl 2,5%, NaOCl 5%, dan EDTA 17%. Kekerasan mikro dentin saluran akar diukur dengan Vickers Hardness Tester sebelum dan setelah perendaman. Data hasil penelitian dianalisis dengan uji One Way ANOVA. Hasil penelitian menunjukkan bahwa propolis 8% memiliki nilai penurunan kekerasan mikro dentin yang paling kecil dari seluruh kelompok perlakuan dengan selisih rerata kekerasan mikro dentin awal dan akhir yaitu 3,68 diikuti oleh propolis 20%, NaOCl 2,5%, NaOCl 5%, propolis 30% dan

EDTA 17%. Kesimpulan dari penelitian ini yaitu terdapat perbedaan efek yang signifikan (p<0,05) dari masing-masing kelompok perlakuan terhadap kekerasan mikro dentin saluran akar. Ekstrak propolis 8% dan 20% dapat digunakan sebagai bahan irigasi alternatif.

***Kata kunci**: bahan irigasi; EDTA; kekerasan mikro; NaOCl; propolis*

INTRODUCTION

Irrigation plays a crucial role in the preparation process of root canals, as this stage cleanses the root canal tissue that endodontic instruments cannot directly access.[1] Irrigating solutions have several ideal requirements, including having sufficient solubility to clean necrotic tissue and bacteria within the root canal without damaging the dentin structure of the root, having the ability as a lubricant, not causing discoloration of the dental tissue and not toxic.[2]

The microhardness of root canal dentin can be used to determine whether the dentin encounters a decrease or increase in mineral content. A decrease in the microhardness of dentin can facilitate root canal instrumentation; however, this condition can potentially weaken the tooth's overall structure.Three irrigating materials with varying concentrations and durations can alter the ratio of organic and inorganic components in dentin. They could also change the root canal dentin's microhardness and increase the coronal leakage risk.[4]

Sodium hypochlorite (NaOCl) is an irrigant that effectively dissolves tissue, including necrotic tissue and vital pulp tissue.[4] Therefore, sodium NaOCl is the most commonly used irrigant, with concentrations ranging from 1% to 5.25%.[5] Although widely used, NaOCl still has several adverse effects. Some of these include unpleasant taste and odor, inability to remove the smear layer, potential allergenic reactions in some patients, and discoloration of clothing.[6] Since NaOCl can only dissolve organic tissue, another irrigating solution is needed to remove the smear layer in the root canal, such as EDTA.[7] EDTA solution is generally used at a concentration of 17%. Applying 17% EDTA for one minute using ultrasonic techniques effectively removes the smear layer, particularly in the apical third of the root.[7,8]

Herbal products are extensively researched due to their potential applications in various fields.[9] In recent

years, propolis has attracted the attention of researchers due to its potential as an alternative irrigant.[10] Propolis is a natural resin compound collected by bees from various types of plants.[11] Propolis comprises plant resin (60%), pollen, and wax (30%). Flavonoids, phenolic acids, and terpenoids are the main components of propolis that contribute as active agents in activities such as inhibiting microbial growth, anti-inflammatory reactions, and antioxidant properties.[12] Propolis has antibacterial properties similar to sodium hypochlorite (NaOCl), the most commonly used irrigating solution.[13] The study by Yuanita (2017) showed that propolis at a concentration of 8% could clean the smear layer more effectively than sodium hypochlorite at concentrations of 2.5% and 5%.[14] The research by Saxena *et al.* reported that propolis exhibited a larger zone of inhibition against *E. faecalis* compared to other herbal extracts after sodium hypochlorite.[15]

This *in-vitro* study aims to evaluate the differences in the root canal dentin microhardness using propolis extracts, NaOCl, and EDTA as irrigants.

METHOD

Sample preparation

Twenty-four extracted human permanent premolars with fully formed, single, and straight roots were selected. The teeth with multiple roots, caries, cracks, and restoration were excluded. The crowns were removed at the cementoenamel junction from each tooth using a low-speed diamond disk bur, and the pulp tissue was removed using a barbed broach. Each root was sectioned parallel to the tooth axis from the buccal to the lingual side using a low-speed diamond disk bur under running water. The samples were embedded in a PVC mold using resin acrylic self-cure with the dentin surface facing upward. Samples were numbered 1-48 randomly and divided into six groups (n = 8). Group 1 consisted of samples number 1-8, Group 2: samples number 9-16, Group 3: samples number 17-24, Group 4: samples number 25-32, Group 4: samples number 25-32, Group 5: samples number 33-40, Group 6: samples number 41-48. Microhardness values of root canal dentin were recorded before immersion using a Vickers hardness tester with 200g indenter load and 20 s dwell time.

Propolis extract preparation

Two kilograms of propolis were blended and extracted using 70% ethanol through a maceration method. After five days, the maceration was stopped, and the ethanol extract of propolis was evaporated to remove any remaining ethanol liquid. The evaporation of the propolis ethanol extract was carried out until a concentrated

propolis extract (100%) was obtained. The ethanol extract of propolis was then diluted using distilled water to obtain propolis extracts with concentrations of 8%, 20%, and 30%, each in a volume of 100 ml.

Treatment of samples

Each group of samples was immersed in irrigant solutions as the following:

Group 1: Immersed with 8% propolis for 5 minutes.

Group 2: Immersed with 20% propolis for 5 minutes.

Group 3: Immersed with 30% propolis for 5 minutes.

Group 4: Immersed with 2.5% NaOCl for 5 minutes.

Group 5: Immersed with 5% NaOCl for 5 minutes.

Group 6: Immersed with 17% EDTA for 5 minutes.

All samples were rinsed using saline solutions after being immersed and dried using a chip blower.

Measurement of the root canal dentin microhardness after treatment

The microhardness values of each sample were measured again using the same method as the initial microhardness measurement. The results were recorded as the microhardness values of dentin after treatment.

RESULT

The data was collected using the Statistical Package for the Social Sciences (SPSS). The data were analyzed using the Shapiro-Wilk test to detect whether the data were normally distributed or not. Differences in the root canal dentin microhardness between each group are performed using one-way analysis of variance (ANOVA) if the data are typically distributed and using Kruskal-Wallis if it is not.

Table 1. The decreased percentage before and after treatment of the microhardness values in each group

Group	Percentage decrease (Mean ± SD)
8% Propolis	3.68 ± 0.48
20% Propolis	7.17 ± 0.53
30% Propolis	10.27 ± 1.09
2.5% NaOCl	9.00 ± 0.35
5% NaOCl	11.48 ± 0.49
17% EDTA	14.63 ± 0.58

All sample groups encounter decreased root canal dentin microhardness, with varying differences in the mean microhardness (VHN) values.

Table 2. Shapiro-Wilk test of the microhardness values in each group

Group	P-Value (Shapiro-Wilk)
8% Propolis	0.25
20% Propolis	0.20
30% Propolis	0.18
2.5% NaOCl	0.23
5% NaOCl	0.22
17% EDTA	0.23

The results of the Shapiro-Wilk normality test indicated that the data on the change in microhardness of root canal dentin after immersion in 8% propolis, 20% propolis, 30% propolis, 2.5% NaOCl, 5% NaOCl, and 17% EDTA for 5 minutes were usually distributed ($p>0.05$).

Table 3. One-way ANOVA test of the changes in microhardness values in each group

Group	n	Mean	P-Values (ANOVA)
8% Propolis	8	3.68	
20% Propolis	8	7.17	
30% Propolis	8	10.27	
2.5% NaOCl	8	9.00	
5% NaOCl	8	11.48	0.001
17% EDTA	8	14.63	

The results of the ANOVA test showed a p-value = 0.001 < 0.05, indicating that the null hypothesis (H_0) is rejected. There is a significant difference between the effects of 8%, 20%, and 30% propolis, 2.5% and 5% sodium hypochlorite, and 17% EDTA on the microhardness of root canal dentin.

DISCUSSION

The level of mineral content and the quantity of hydroxyapatite in the intertubular substance are essential factors in assessing the intrinsic hardness characteristics of dentin structure.[5] In this study, the treatment of immersing samples in 8% propolis extract for 5 minutes resulted in a minor decrease in the microhardness of dentin among all groups, with a difference in the average initial and final microhardness values of 3.68. Followed by the irrigating materials of 20% propolis extract, 2.5% NaOCl, 30% propolis extract, and 17% EDTA.

The treatment with 17% EDTA irrigation for 5 minutes resulted in the greatest decrease in dentin microhardness among all groups, with a difference in the average initial and final microhardness values of 14.63. It is consistent with the research by Aslantas et al. (2014), which also demonstrated that immersing samples in 17% EDTA for 5 minutes significantly reduced the microhardness of root canal dentin, with a similar difference in values of 14.45.[4] This effect occurs because EDTA is

a chelating agent that can dissolve inorganic components in the root canal by binding to calcium ions, which can weaken parts of the root canal dentin. Therefore, this leads to a decrease in the microhardness of root canal dentin while also facilitating the dissolution of the smear layer.[3,5,16]

Similarly, in this study, the research by Kandil et al. (2014) showed that immersing samples for 5 minutes in 2.5% NaOCl resulted in a smaller decrease in the microhardness of root canal dentin compared to 17% EDTA. Sodium hypochlorite (NaOCl) has organic solvent properties that can affect the collagen components in dentin. The irrigation process with NaOCl, at concentrations ranging from 2.5% to 5.25%, can reduce the collagen content in dentin, impacting root canal dentin's microhardness.[3] The higher inorganic content of root canal dentin (70%) compared to the organic content (20%) is why EDTA can lead to a more significant decrease in microhardness than NaOCl, which only dissolves organic components in the root canal.[17]

Many studies have been carried out to evaluate the potential of propolis as an irrigant. Research by Bhuvnesh *et al.* (2019) reported that propolis at concentrations of 5% and 20% is effective against *S. mutans* and *L. acidophilus*.[18] Additionally, a study by Awawdeh *et al.* (2018) demonstrated that a 30% propolis extract has effectiveness comparable to chlorhexidine and 3% NaOCl against *C. albicans*.[19] Furthermore, research by Yuanita (2017) indicated that an 8% propolis extract could clean the smear layer more effectively than 2.5% and 5% NaOCl.[14] Kalyoncuoğlu et al. (2015) reported that a 20% propolis extract used as a final irrigating material can enhance the bond quality between self-etch adhesives and the root canal dentin surface.[10] These findings support the use of propolis extract as an alternative irrigant.

In this study, propolis extracts at concentrations of 8%, 20%, and 30% were tested for their effects on the microhardness of root canal dentin, with the 8% propolis extract showing a minor decrease in microhardness, followed by the 20% propolis extract. The 30% propolis extract is in fourth place after 2.5% NaOCl.

Phenolic acid esters found in propolis are weak acids acting as chelating agents (binding metal ions).[20] This indicates that these weak acids share a mechanism similar to EDTA. The weak acids attach to the dentin structure, leading to an equilibrium reaction between the weak acids and hydroxyapatite in the root canal dentin. Once bound, these acids release hydrogen ions (H^+) from hydroxyapatite. The hydrogen ions (H^+) can displace calcium ions from the hydroxyapatite

crystal structure, reducing mineral content.[16,21] This equilibrium reaction can affect the properties of root canal dentin, including microhardness, fracture resistance, and increased permeability, among others.

CONCLUSION

Through this study, it can be concluded that the root canal dentin microhardness differences exist in each treatment group. Propolis with 8% and 20% concentration can be used as safer alternative irrigants in terms of reducing the microhardness of root canal dentin compared to 2.5% and 5% NaOCl, as well as 17% EDTA.

CONFLICT OF INTEREST

We declare no potential conflict of interest in the articles we write.

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